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(54) Title: ACYLATED ANTIMICROBIAL PEPTIDES

(57) Abstract: Acylated peptides which exhibit antimicrobial activity comparable to certain known antibiotics are provided. These peptides are related in sequence to amino acid sequences within Cathepsin G. A broad spectrum acylated bactericidal peptide disclosed herein is PRGTLCTVAGWGRVSMRRGT (amino acids 117-136 of SEQ ID NO:1); it is active against *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Staphylococcus aureus*. The antibacterial activity is enhanced by acylation at either the N- or the C-terminus with a fatty acid of from 2-20, desirably 8-14 carbon atoms. Additional antimicrobial and acylated antimicrobial peptides are disclosed. The acylated and/or hydrophobic antimicrobial peptides of the present invention are useful in pharmaceutical compositions useful in the treatment or prophylaxis of infections or in compositions to inhibit microbial growth.

ACYLATED ANTIMICROBIAL PEPTIDES

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BACKGROUND OF THE INVENTION

The field of this invention is the area of antimicrobial peptides with activity against a broad range of Gram negative and Gram positive bacteria and fungi, in particular, where those peptides are acylated such that antimicrobial activity is significantly improved. The antimicrobial peptides of this invention are useful for inhibiting microbial growth and in pharmaceutical compositions for treatment or prevention of infections.

Microbes which invade the human body are challenged by several defense mechanisms. The nature of the defense mechanisms which any given microbe faces depends on the genetic makeup and the physiologic state of the host as well as the portal of entry of the invading microorganism.

If the mechanical and chemical barriers of the skin or mucous membranes are crossed, immunological factors (e.g., antibodies) which are specific to the microorganism and nonspecific cellular defenses come into play. Nonspecific cellular defenses in the form of phagocytic white blood cells from local tissues and the bloodstream respond to an invading microbe. Polymorphonuclear leukocytes (PMNs) actively phagocytize particulates such as bacterial or fungal cells. PMNs are the first class of phagocytic cells recruited to the site of

infection or inflammation, and their primary function is to ingest and kill microorganisms via oxygen-dependent and oxygen-independent mechanisms.

Our research program has emphasized the oxygen-independent process since PMNs often kill pathogens at hypoxic sites [2]. Moreover, in certain patients (e.g., those patients suffering from chronic granulomatous disease) who have defects in their oxygen-dependent killing system, the oxygen-independent killing processes carry the burden of PMN killing of phagocytosed bacteria [5].

The cationic proteins and peptides stored within the cytosolic granules of PMNs are important mediators of oxygen-independent killing of bacteria [2, 5]. They belong to an increasingly growing list [6-19] of antimicrobial peptides that have been isolated from fluids and extracts obtained from a variety of sources including PMNs from other vertebrates, from other mammalian cells, invertebrates, and plants. A historical account of the discovery of cationic proteins from various vertebrate sources has been provided [2,5].

Because of their antibiotic-like action, in recent years CAPs derived from various sources have received significant attention as futuristic therapeutic agents in the treatment of microbial infections and/or sepsis and in agricultural applications [14,18]. Interest in these compounds has been spurred by the increasing problem of multi-drug resistant pathogens and the relative dearth of new and effective antibiotics available to treat certain infections.

Predominant CAPs within human PMNs include lactoferrin [22,23], bactericidal/permeability increasing protein (BPI) [24], CAP37/azurocidin [25-29], Cat G [30,31], elastase [33], lysozyme [2], LL-37 derived from CAP 18 [34,35] and defensins [36]. These CAPs are delivered into the maturing phagolysosome by the process of degranulation [5] where they can reach high concentrations (e.g., mg/ml). Alternatively, some CAPs (e.g., LL-37) are secreted to the extracellular fluid where they can exert their antimicrobial action or perform other biologic functions, such as binding endotoxin [36]. The multitude of CAPs is likely to reflect the need to kill different pathogens under different environmental conditions such as pH and salt concentrations. These PMN-derived CAPs along with those CAPs (β-

defensins) secreted by certain epithelial cells [8,9,12] that line mucosal surfaces are likely to be of importance in host defense before specific humoral and cellular defenses become available. Their contribution to host defense is believed to reduce the microbial load to a level that would permit greater effectiveness by subsequent defense systems.

Cat G is a highly cationic (pI > 12), arginine-rich, neutral serine protease found in the azurophilic granules of PMNS [38]. The complete amino acid sequence of Cat G (Fig. 1, SEQ ID NO:1) was reported by Salvesen et al. [39]. They recognized that Cat G is similar to granzyme B (produced by activated human T-cells) and mast cell chymase. Cat G consists of at least three isoforms that are generated due to differences in glycosylation and *C*-terminal processing [39]. The enzymatic activity of Cat G resembles chymotrypsin. The arginine-rich property of Cat G is remarkable and is probably an important determinant of its bactericidal action.

Besides its potent activity against gonococci, our interest in Cat G has been fueled by a number of observations. First, against certain pathogens, Cat G is the most potent antimicrobial protein thus far isolated from human PMNs. Second, against other pathogens (e.g., *S. aureus*, and *Listeria monocytogenes*), enzymatically-inactive Cat G is as bactericidal as enzymatically-active Cat G [30,31,49,50], while against other pathogens (*Capnocytophaga sputigena*), only enzymatically active Cat G is bactericidal [51,52]. Initially, we thought that this "protease requirement" reflected different modes of killing that could be carried out by full-length Cat G. However, we subsequently determined that synthetic peptides derived from Cat G could mediate killing of pathogens, including those that could only be killed by enzymatically-active Cat G.

Because Cat G can undergo auto-proteolysis and self-digested preparations retained bactericidal action, we hypothesized that Cat G peptides possessing bactericidal activity could be isolated. Cat G was digested to completion with a bacterial protease [53]. The digested Cat G preparation remained bactericidal against *N. gonorrhoeae, P. aeruginosa* and *S. aureus*. Fractionation of this sample resulted in the recovery of two small peptides that represented amino acyl residues 1-5 of SEQ ID NO:1 (IIGGR) and amino acids 77-83 of SEQ ID NO:1

(HPQYNQR) of full-length Cat G. However, the combined bactericidal action of these peptides was less than 5% of full-length Cat G, suggesting that other bactericidal regions of Cat G had been lost during protease digestion. Interestingly, however, human granzyme B from activated cytolytic T lymphocytes (CTLs) has a peptide similar to HPQYNQR at a nearly identical position within its primary sequence. A synthetic peptide (HPAYNPK) corresponding to the homologous granzyme B [54] peptide sequence also exhibited bactericidal activity in vitro [55]. This observation suggested to us that CTLs could also participate in nonoxidative killing of bacteria or that they might use this sequence along with perforin for their cytolytic action.

In order to identify other antibacterial regions within Cat G, we prepared non-overlapping peptides of 20 amino acids and tested their activity against *P. aeruginosa* and *S. aureus* [56]. We found four peptides that possessed varying degrees of bactericidal activity *in vitro*. One of these peptides (CG 1-20), representing the *N*-terminal twenty amino acids of Cat G, is likely buried (based on X-ray crystallographic data [57]) within the Cat G molecule, while other antibacterial peptides (CG 61-80, 117-136 and CG 198-223) appear to be largely localized on the surface of the Cat G protein where they could easily interact with microbial surfaces.

Of the four major antibacterial peptides identified within the full-length Cat G molecule, only one (CG 117-136) had activity [56,58] against all of the major pathogens tested (*N. gonorrhoeae, P. aeruginosa,* and *S. aureus*). In addition to its capacity to exert antibacterial action against these pathogens, the CG 117-136 peptide was also active against certain oral pathogens implicated as agents of periodontal disease (*Actinobacillus actinomycetemcomitans* and *C. sputigena*).

Analysis of the CG 117-136 peptide's sequence (Fig. 1, SEQ ID NO:1) revealed that it is an amphipathic peptide with an *N*-terminal hydrophobic domain and a *C*-terminal cationic domain. Briefly, the modeled structure predicts the guanidino groups of arginine, which are clearly important for bactericidal activity [58], are surface-exposed and not electrostatically neutralized by counter ions from the rest of the protein. The structure predicts that within

peptide CG 117-136, two surface-exposed regions would exist; these correspond to ^{117}RPGTLC^{122} (amino acids 117-122 of SEQ ID NO:1) and ^{129}RVSMRRGT^{136} (amino acids 129-136 of SEQ I D NO:1; underlined in Figure 1). Thus, we believe that in full-length Cat G, the major antibacterial peptide is largely exposed to the solvent. In order to test whether just the surface-exposed regions of CG 117-136 are sufficient for bactericidal activity, we prepared a hybrid peptide that linked amino acids117-122 to 129-136 through a six-carbon linker; the six-carbon linker was chosen to mimic the distance between these peptides afforded by residues 123-128. We found that at 50 μg/ml of either the wild-type peptide or the hybrid variant, greater than 95% of the input *P. aeruginosa* bacteria were killed.

As described in WO 91/04414, the Cat G protein was analyzed to determine whether the same portions of the protein were responsible for the enzymatic and antibacterial activity. Purified human Cat G was digested with the proteolytic enzyme clostripain. Peptides resulting from that digestion were purified and individually tested for antibacterial and enzymatic activity. None of the peptides tested exhibited the chymotrypsin-like activity of the intact molecule, but two Cat G-derived peptides exhibited antibacterial activity using *Staphylococcus aureus* or *Neisseria gonorrhoeae* as indicator organisms: IIGGR (amino acids 1-5 of SEQ ID NO:1) and HPQYNQR (amino acids 77-83 of SEQ ID NO:1). Antimicrobial activity was maintained with some variation in amino acid sequence, as described in WO 91/04414. Similarly, the oligopeptide corresponding in amino acid sequence to amino acids 1-20 of Cat G exhibited strong bactericidal activity against *Pseudomonas aeruginosa*.

Similarly, WO 94/07523 shows that certain twenty amino acid peptides corresponding in sequence to portions of the Cat G protein had significant antimicrobial activity in vitro as well as in vivo. The most active of those peptides tested were CG 1-20, CG 117-136 and CG 198-223.

There is a long felt need in the art for new and effective antimicrobial agents, especially in view of the development of widespread and multiple antibiotic resistance phenotypes in the etiological agents of human and animal disease.

SUMMARY OF THE INVENTION

It is an object of this invention to provide acylated oligopeptides with improved antimicrobial activity, as compared to the corresponding oligopeptides which have not been acylated. The acylated antimicrobial oligopeptides of the present invention contain from five to about twenty-seven amino acids joined in a linear array by peptide bonds, preferably from ten to about twenty-six amino acids. The amino acids can be all L configuration, all D configuration or a combination of configurations. The acyl moiety contains from about 2 to about 20 carbon atoms, desirably from about 8 to about 14, and advantageously containing 10 or 12 carbon atoms. The acyl moiety be a saturated fatty acid, and can be straight chain or branched, or the acyl moiety can be an unsaturated fatty acid. Acylation can be at the N-terminus of the peptide, or where the peptide has as its C-terminus an amino acid with a free (epsilon) amino group, desirably lysine, acylation can be at the C-terminus.

An object of the present invention is an acylated broad spectrum bactericidal oligopeptide, termed acyl-CG 117-136 herein, which has the sequence RPGTLCTVAGWGRVSMRRGT (amino acids 117-136 of SEQ ID NO:1) or RPGTLCTVAGWGRVSMRRGTK (SEQ ID NO:14). The present invention includes the acyl-CG 117-136 oligopeptide in which all correspondent amino acids are L-amino acids (L-enantiomer) and the acyl-CG 117-136 peptide in which some or all the component amino acids are D-amino acids (D-enantiomer). Further objects are additional acylated bactericidal oligopeptides, termed acyl-CG 122-136 (amino acids 122-136 of SEQ ID NO:1), acyl-CG 127-136 (amino acids 127-136 of SEQ ID NO:1), acyl-CG 61-80 (amino acids 61-80 of SEQ ID NO:1) and acyl-CG 198-223 (amino acids 198-223 of SEQ ID NO:1), herein. Additional antimicrobial oligopeptides include those having the amino acid sequences given, e.g., in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7. For all of these peptides, where C-terminal acylation is desired, there is an added lysine as the C-terminal amino acid to provide a free amino group for acylation. These sequences are given in SEQ ID NO:8-14.

In other embodiments, the antimicrobial oligopeptides comprise the amino acid sequence of acyl-CG 1-20 (amino acids 1-20 of SEQ ID NO:1). Acyl-CG 1-20 has antimicrobial activity against bacteria including, but not limited to, *Pseudomonas aeruginosa* and oral pathogens such as *Capnocytophaga sputigena*, *Eikonella corrodens* and *Actinobacillus actinomycetemcomitans*.

An object of the present invention is to provide acylated antimicrobial oligopeptides which are useful as antibiotics, bactericides and/or bacteriostats, useful, for example, for killing microorganisms or for inhibiting microbial growth in a variety of solutions and sterile solutions, such as contact lens solutions, herbicidal solutions, hazardous or refuse waste streams, surface disinfectant solutions and oil recovery fluids, and/or are effective for the treatment of bacterial infections.

A further object of the invention is to provide therapeutic compositions, suitable for human, veterinary, agricultural or pharmaceutical use, comprising one or more of the antimicrobial acylated oligopeptides of the present invention and a suitable pharmacological carrier. Such therapeutic compositions can be formulated as understood in the art, e.g., for topical or aerosol application, for controlling and/or preventing infection by Gram positive or Gram negative bacteria or fungi. Preferably, the antimicrobial oligopeptides of the present invention are used in the treatment of infections by Gram negative or Gram positive bacteria. In vitro antimicrobial activity of the oligopeptides of the present invention is an accurate predictor of in vivo antimicrobial activity.

Pharmaceutical compositions contain a therapeutically effective amount of an acylated antimicrobial oligopeptide. A therapeutically effective amount of an antimicrobial oligopeptide can be readily determined according to methods known in the art. Pharmaceutical compositions are formulated to contain the therapeutically effective amount of an antimicrobial oligopeptide and a pharmaceutically acceptable carrier appropriate for the route of administration (topical, gingival, intravenous, aerosol, local injection) as known to the art. For agricultural use, the composition comprises a therapeutically effective amount of an antimicrobial oligopeptide and an agriculturally acceptable carrier suitable for the organism

(e.g., plant) to be treated. Preferably for use in a pharmaceutical composition, the acylated antimicrobial oligopeptide will have an ED_{50} in vitro less than about 10^{-3} M. The skilled artisan can readily determine a therapeutically effective amount against a target bacterial strain, for example, based on the ED_{50} using the methods disclosed herein and the teachings of the art.

Therapeutic compositions may be administered by topical, dental rinse, aerosol or intravenous application, or by local injection for the control or prevention of infection, by any means known to the art.

N-terminal acylated CG 1-20 (amino acids 1-20 of SEQ ID NO:1) or the C-terminal acylated oligopeptide of SEQ ID NO:14 can also be used to kill or control the growth of tumor cells or virus-infected cells. In such applications, these peptides will be particularly useful when coupled to antibodies or other molecules which are specific for the target tumor cell or virus-infected cell so that the peptide acts specifically on the tumor or virus-infected cell.

In the context of the present invention, acylated means that a fatty acid molecule is covalently linked to an antimicrobial oligopeptide. As specifically exemplified, the fatty acid is covalently linked to the N-terminus of the oligopeptide or it is covalently linked to the C-terminus of oligopeptide. Desirably the fatty acid moiety is from 2 to about 20 carbons in length, and the fatty acid is a saturated fatty acid but unsaturated fatty acids can also be used to increase antimicrobial activity. Desirably the fatty acid moiety is a straight chain alkyl acid, but branched chain, saturated straight chain or branched chain hydroxylated, and branched chain or straight unsaturated fatty acids, with or without hydroxylation, can also be used in the practice of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows, in the one letter code, the complete amino acid sequence of human lysosomal Cat G (see also SEQ ID NO:1). The antibacterial peptide defined by residues 117-136 is highlighted in bold. The two internal peptides of CG 117-136 that are exposed on the surface of Cat G are underlined.

Figure 2 demonstrates the impact of covalent attachment of saturated fatty acids to the *N*-terminus of CG 117-136 on bactericidal activity. Values are expressed as $\log_{10}$ bactericidal units when the modified peptides were tested at 50 μg/ml against *S. aureus* strain 37533.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, an oligopeptide is composed of from about five to about twenty-seven amino acids linked together by peptide bonds in a linear array. The peptide may be in a linear conformation or it may assume secondary structure. A cyclic peptide derivative can also have antimicrobial activity, and thus is a functional equivalent of the antimicrobial peptides of the present invention. Sequences are conventionally given from the amino terminus to the carboxyl terminus. Component amino acids may be of the D- or the L-configuration. Unless otherwise noted, the amino acids are L-amino acids. When all component amino acids are of L-configuration, the peptide is said to be an L-enantiomer. When all the amino acids in a peptide are in the D-configuration, that peptide is said to be a D-enantiomer. The peptides of the present invention have significantly improved antimicrobial activity when coupled to a fatty acid moiety, either at the N-terminus or the C-terminus of the oligopeptide.

Abbreviations used in this application include A, Ala, Alanine; M, Met, Methionine; C, Cys, Cysteine; N, Asn, Asparagine; D, Asp, Aspartic Acid; P, Pro, Proline; E, Glu, Glutamic Acid; Q, Gln, Glutamine; F, Phe, Phenylalanine; R, Arg, Arginine; G, Gly, Glycine; S, Ser, Serine; H, His, Histidine; T, Thr, Threonine; I, Ile, Isoleucine; V, Val, Valine; K, Lys, Lysine; W, Try, Tryptophan; L, Leu, Leucine; Y, Tyr, Tyrosine; Boc, tert-butyloxycarbonyl; CFU, colony forming unit; DFP, diisopropylfluorophosphate; HLE, human leukocyte elastase; Pam, (phenylacetamido) methyl; CAPs, cationic antimicrobial proteins/peptides; CAP 18, cationic antimicrobial protein of 18,000 daltons; CAP 37, cationic antimicrobial protein of 37,000 daltons; Cat G, cathepsin G; DFP, diisopropyl fluorophosphate; kDa, kilodaltons; LL-37 = 37 amino acid antibacterial peptide derived from CAP 18 having leucine as the first two *N*-terminal amino acids; LPS, lipopolysaccharide; PG, peptidoglycan; PMN, polymorphonuclear leukocyte.; BSA, bovine serum albumin. Other abbreviations are as commonly used in the art.

ED_{50} is the concentration of an antimicrobial agent which kills (or otherwise inhibits growth) 50% of the input indicator microorganisms or cells under particular test conditions.

MIC (minimal inhibitory concentration) is the concentration required to prevent growth of bacterial cells in liquid medium.

For convenience, the peptides disclosed herein are named according to the amino acid positions in mature Cat G (Fig. 1).

CG 1-20 represents amino acid residues 1-20 of the mature Cat G sequence and has the sequence IIGGRESRPHSRPYMAYLQI (amino acids 1-20 of SEQ ID NO:1).

CG 61-80, corresponding to amino acids 61-80 of Cat G, has the sequence RRENTQQHITARRAIRHPQY (amino acids 61-80 of SEQ ID NO:1).

CG 117-136, corresponding to amino acids 117-136 of Cat G, has the sequence RPGTLCTVAGWGRVSMRRGT (amino acids 117-136 of SEQ ID NO:1).

CT 198-223, corresponding to amino acids 198-223 of Cat G, has the sequence GKSSGVPPEVFTRFVSSFLPWIRTTMR (amino acids 198-223 of SEQ ID NO:1)

Antimicrobial activity, as used herein, refers to the ability of an acylated peptide of the present invention to kill at least one species selected from the group consisting of Gram positive bacteria, Gram negative bacteria, fungi, and protozoans. It is increasingly preferred that the peptide kill at least 50%, 60%, 70%, 80%, 90% or all cells of at least one species of Gram positive or Gram negative bacteria, fungi, or protozoans. Sensitive Gram positive bacteria can include, but are not limited to, *Staphylococcus aureus*, *Streptococcus pneumoniae* and Enterococci. Sensitive Gram negative bacteria include, but are not limited to, *Escherichia coli*, *Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa*. Periodontal disease-associated bacteria include *Capnocytophaga sputigena*, *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens*. Sensitive fungi can include, but are not limited to, *Candida albicans*.

Antimicrobial activity can also refer to the ability to kill or inhibit the growth of other cells, in particular, those which are tumor cells or virus-infected cells.

The acylated antimicrobial peptides of the present invention are oligopeptides which possess antimicrobial activity, as defined herein. Modified peptides with antimicrobial activity are functionally equivalent to the antimicrobial peptides of the present invention.

Antibacterial pharmaceutical compositions, as defined herein, comprise a pharmaceutically acceptable carrier and one or more antibacterial peptides of the present invention. Such antimicrobial pharmaceutical compositions may be formulated in ways, as understood in the art, for use for topical application, for gingival application (for gingivitis or periodontal disease) or for local or systemic injection. For use in the treatment or prevention of gingivitis, the peptides of the present invention can be incorporated in effective amounts in a dental rinse for application to the buccal area, or they may be incorporated in other suitable compositions for topical application. The antibacterial peptides of the present invention may also be incorporated in effective amounts in chewing gum, lozenges for sucking, toothpowder or toothpaste. The antibacterial peptides of the present invention can comprise from 0.001% to 50% by weight of such compositions. It will be understood that a composition for systemic injection will contain an antimicrobial peptide, e.g., an antibacterial peptide such as CG 117-136, in a therapeutically effective amount or a therapeutically effective amount of an antimicrobial peptide can be conjugated to an antibody, or any other compound as understood in the art, with specificity for the target cell type. The choice of the peptide will be made with consideration of immunogenicity and toxicity to the infected host, effective dose of the peptide, and the sensitivity of the target microbe to the peptide, as well-understood in the art.

Of the four antibacterial Cat G peptides that we identified through the synthetic peptide chemistry approach, only CG 117-136 exerted activity against both *P. aeruginosa* and *S. aureus* [56]. Due to its broad-spectrum of activity, we have focused our efforts on CG 117-136 so as to learn not only how it contributes to the bactericidal action of Cat G, but more importantly, how it might serve as a model for how other human cationic peptides exert

bactericidal action. CG 117-136 contains an *N*-terminal hydrophobic region and a *C*-terminal cationic region.

In order to learn if both regions contribute to its bactericidal action, peptides with – or *C*-terminal truncations were prepared and tested for their capacity to kill *P. aeruginosa* and *S. aureus*. Such truncated peptides were, on a molar basis, less active than the full-length peptide [58]. We concluded that the killing activity of CG 117-136 required the contributions of both regions, with the cationic section promoting ionic interactions with the microbial surface and the hydrophobic section being important for membrane insertion. The importance of ionic interactions of CG 117-136 with the bacterial surface was confirmed in binding experiments. We found that surface-binding decreased with increasing concentrations of NaCl or divalent cations in the binding assay broth. In order to learn if CG117-136 might recognize a specific microbial target with a chiral center, an all D-amino acid variant was synthesized and tested against both pathogens. This seemed not to be the case because the D-enantiomeric peptide was as active as the L-enantiomer [56].

Alanine-scanning was used to identify residues of CG 117-136 that are critical for its broad spectrum antibacterial action. Synthetic peptide variants containing single alanine substitutions at 19 of 20 residues were prepared and tested for bactericidal action against *P. aeruginosa* and *S. aureus*. Replacement of any of four arginine residues (at positions 117, 129, 133 and 134 in the full-length Cat G primary sequence) or certain hydrophobic residues (Leu-121, Cys-122, Val-124, Trp-127) with alanine resulted in peptides with significantly (two to four log reduction) diminished bactericidal activity. In contrast, replacement of the wild-type alanine at position 125 with arginine resulted in a peptide with enhanced bactericidal activity compared to parental peptide CG 117-136 [58]. These results confirm the importance of the cationic residues in the binding of the peptide to the negatively charged bacterial surface while the nonpolar residues are needed for insertion into the hydrophobic bacterial membrane.

The importance of arginine residues in determining bactericidal activity was further evident by the diminished bactericidal activity of synthetic peptides containing either lysines or citrullines instead of arginines [58]. Moreover, introduction of a single negatively charged

amino acid (glutamic acid) also diminished the bactericidal action of CG 117-136. We concluded from these studies that while cationicity due to the guanidinium group of arginine is an important determinant in the bactericidal action of CG 117-136, the inability of lysine-bearing peptides to exert wild-type levels of antibacterial action likely reflects the role of hydrogen-bonding potential of the guanidinium side chain of arginine. The guanidinium side chain of arginine is an important structural component in determining activity and its contribution cannot be replaced by the side chains of lysine or citrulline. Each arginine side chain has a maximum potential of donating five hydrogen bonds in contrast to the three for the side chains of lysine and citrulline. The data indicate that both the cationic and hydrogen-bonding potential of the guanidinium side chain are important in peptide-microbial target(s) interactions. It is noteworthy that the lysosomal CAPs Cat G [39] and CAP37 [59] are high in arginine content (33 of 223 residues for Cat G and 22 of 221 for CAP 37) and low in lysine content (three in Cat G and one in CAP 37).

We recently tested whether increasing the hydrophobic characteristic of CG 117-136 would enhance its bactericidal activity. Because we did not wish to alter the amino acid sequence of CG 117-136, we conjugated saturated fatty acids of various lengths (4-18 carbons) to the *N*-terminal free amino group of arginine to increase the hydrophobicity of the peptide. As is shown in Fig. 2, peptides with C-10 (decanoyl) or C-12 (dodecanoyl) fatty acid attachments were surprisingly more active than the nonacylated CG 117-136 or peptides with shorter or longer fatty acid attachments. Similar data were obtained with peptides having the C-10 and C-12 fatty acids attached to the epsilon amino group of the C-terminus lysine added to the end of the CG 117-136 peptide. The increased (10- to > 100- fold) bactericidal activity of the C-12-modified peptide was noted when it and the CG 117-136 peptide were tested against a panel of antibiotic-resistant Gram-positive pathogens (methicillin-resistant *S. aureus*, multi-drug resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus spp.* See Table 1. In other experiments with Gram-negative bacteria (*E. coli*, *N. gonorrhoeae*, and *P. aeruginosa*), which are intrinsically more sensitive to CG 117-136 than Gram-positive bacteria, we observed that the C-12 acylation of the *N*-terminus of CG 117-136 also enhanced bactericidal activity. We stress that the bactericidal action of the C-12 modified peptide required both the fatty acid and the primary amino acid sequence of CG 117-136 because

nonbactericidal peptides of Cat G, representing amino acids of 157-176 and 177-196 of the Cat G protein, similarly modified by C-12 acylation, remained inactive. Accordingly, we propose that the acylation of an antimicrobial peptide derived in sequence from Cat G, for example with a C-12 saturated fatty acid, enhances membrane insertion of the peptide and that this results in increased bactericidal activity.

We have investigated the behavior of the CG 117-136 and the C-12 (lauryl) modified variant in fluids that mimic membrane environments. The objective was to determine whether the covalent attachment of the hydrophobic fatty acid to the N-terminal amino group affects the capacity of CG 117-136 to form an α-helical structure.

The native and fatty acid modified (acylated) peptides were each incubated in the presence of distilled water, trifluoroethanol (TFE) or in a zwitterionic lipid (phosphatidyl choline). Through analyses of the CD spectra of the peptides, we determined the percentage β-structure and α-helix of these two peptides. In water, both peptides have about 35% β-structure and 0% α-helical content. However, in TFE the C12 peptide variant reached 60% α-helix much faster than the unmodified peptide at either 15% and 50% concentration. In the case of phosphatidyl choline, 60% of helix is reached at 30 lipid/peptide molar ratio for the C-12 peptide, while the unmodified peptide reached only 2% α-helical content. These results suggest that the C-12 modification accelerates or enhances the capacity of the acylated CG 117-136 peptide to form an α-helix in model membranes, which may explain its improved antibacterial activity relative to the nonacylated peptide.

The acylated CG 117-136, which has significant antimicrobial activity, is useful as a therapeutic agent. This acylated peptide was tested for toxicity and lethality in vivo for mice. The LD_{50} of the modified peptide is >50 mg/kg when the peptide is administered intraperitoneally and 20-25 mg/kg when it is given intravenously in female Balb/C mice.

Acylated peptides derived in sequence from CAPs used therapeutically have a high therapeutic index would be most desirable, and they are active against pathogens that have become resistant to multiple antibiotics.

As the CG 117-136 peptide is especially active against diverse bacterial pathogens, including antibiotic resistant strains, we have selected it for further analysis and improvement of antimicrobial activity. We confirmed the importance of cationicity and the role of the arginine residues in antimicrobial action. Arginine functions at the level of ionic and hydrogen-binding to the negatively charged microbial surface, a prerequisite for bactericidal action that seems to be followed by the depolarization of the cytoplasmic membrane [63]. Because antimicrobial peptides must interact with or at least pass through bacterial membranes, we enhanced peptide hydrophobicity by attaching a fatty acid to the *N*-terminus. This strategy has proved successful in the development of a synthetic/modified human antibacterial peptide with substantial *in vitro* activity against antibiotic-resistant pathogens (Table 1). Acylation at the C-terminus is similarly advantageous in the improvement of antibacterial activity.

Therapeutic compositions are formulated to contain from about 0.% to about 90% by weight of at least one (or combination) of the acylated antimicrobial peptides of the present invention, often from about 1% to about 20% or about 1% to about 10%, in a pharmaceutically acceptable carrier. Solid formulations of the compositions for oral administration may contain suitable carriers or excipients such as cornstarch, gelatin, lactose, acacia, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, calcium carbonate, sodium chloride or alginate. Disintegrators that can be used include but are not limited to microcrystalline cellulose, corn starch, sodium starch glycolate and alginate. Tablet binders can include, without limitation, acacia, methylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone, hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose. Useful lubricants for formulation into therapeutic compositions can include magnesium stearates, stearic acid, silicone fluid, talc, waxes, oils and colloidal silica.

Liquid formulations of the therapeutic compositions can include, where oral administration is intended, water or other suspending vehicles and various suspending agents such as methylcellulose, alginates, tragacanth, pectin, kelgin, carrageenan, acacia, polyvinylpyrrolidone and polyvinyl alcohol. The liquid formulations can also include solutions, emulsions, syrups and elixirs containing, together with the active compound(s), wetting agents, sweeteners, and/or coloring and/or flavoring agents. Various liquid and

powder formulations can e prepared by conventional methods for inhalation into the lungs of the person or animal to be treated.

Injectable formulations intended, for example, for intravenous or intraperitoneal use, can contain various carrier such as vegetable oils, dimethylacetamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, polyols (propylene glycol, liquid polyethylene glycol and the like). For intravenous injections, water soluble versions of the compounds may be administered by the drip method, whereby a pharmaceutical formulation of the present invention and a physiologically acceptable excipient is infused. Physiologically acceptable excipients include, without limitation, 5% dextrose, 0.9% saline, Ringer's solution, among others. Formulations for intramuscular injection include suitable salts of the acylated antimicrobial peptides of the present invention which is dissolved and administered in water, 0.9% saline or 5% glucose. A suitable insoluble form of the composition can be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, such as an ester of a long chain fatty acid (e.g., ethyl oleate).

A topical semi-solid ointment formulation typically contains the active ingredient (at least one acylated antimicrobial peptide of the present invention) at a concentration from about 1% to about 20% by weight, in a carrier such as a pharmaceutical cream base. Various formulations for topical use include drops, tinctures, lotions, creams, solutions and ointments containing the active ingredient and various supports and vehicles. The optimal percentage of the active ingredient in each pharmaceutical formulation varies according to the formulation itself and the therapeutic effect desired and the route by which it will be administered.

Conventional methods, well known to those of ordinary skill in the art of medicine or veterinary medicine, can be used to administer the pharmaceutical compositions to a patient. Typically, the pharmaceutical formulation is administered to the patient by applying to the skin of the patient a cream, ointment, lotion or transdermal patch, at intervals appropriate for the condition treated. The pharmaceutical formulations can also be administered via other routes including oral, subcutaneous, intrapulmonary, transmucosal, intraperitoneal, intrauterine, sublingual, intrathecal, or intramuscular routes. In addition, the pharmaceutical formulations

of the present invention can be administered to a human or animal patient by injectable depot routes of administration.

The following examples are provided for illustrative purposes and are not intended to limit the scope of the invention. Because modification of the examples below will be apparent to those of skill in the art, it is intended that this invention be limited only by the scope of the appended claims.

All references cited in this application are hereby incorporated by reference herein to the extent that there is no inconsistency with the present disclosure.

EXAMPLES

Example 1. Preparation of Synthetic Peptides

Oligopeptides were synthesized using an Applied Biosystems Model 430A peptide synthesizer (0.1 - 0.5 mmol scale) using phenylacetamidomethyl (Pam) or p-methylbenzyhydrylamine copoly(styrene/divinylbenzene) resins (Applied Biosystems, Inc., Foster City, CA) and tert-butyloxycarbonyl (Boc)-protected amino acids (Applied Biosystems, Inc. or Bachem, Inc., Torrance, CA). Boc-N-methyl-Ala, Boc-Arg(tosyl) or Boc-Arg(mesitylenesulfonyl), Boc-Asp(benzyl), Boc-Cys(4-methoxybenzyl), Boc-Glu(benzyl), Boc-His(benzyloxycarbonyl) or Boc-His(2,4-dinitrophenyl), Boc-D-His(4-toluenesulfonyl), Boc-Lys(chlorobenzyloxycarbonyl), Boc-Met, Boc-Ser(benzyl), Boc-Thr(benzyl), Boc-Trp or Boc-Trp(formyl), and Boc-Tyr(2-bromobenzyloxycarbonyl) were used for the incorporation of the respective amino acid residues. Boc-His(methyl) was incorporated in a manual mode on a 0.02 mmol scale using the N,N-dicyclohexylcarbodiimide/1-hydroxybenzotriazole coupling protocol. All amino acids (except glycine) used herein have the L configuration unless otherwise noted.

Peptides were cleaved from the resin and deprotected in liquid HF/p-cresol/dimethyl sulfide (10:1:0.5) at -5°C for 90 min, or in liquid HF/anisole (9:1, v/v) at 0°C for 90 min. The resins were washed with cold diethyl ether, and the peptides were extracted into 1.0 M

acetic acid and lyophilized. Peptides were then purified by RP-HPLC on an Aquapore™ RP-300 C18 silica column (1x10 cm, Applied Biosystems, Inc.), or on an MRPH-Gel™ polystyrene column (1 X 10 cm, The Nest Group, Scarborough, MA) using a 0 - 60% linear gradient of acetonitrile in 0.1% TFA. The purity of each synthetic peptide preparation was confirmed by microbore HPLC on Aquapore™ OD-300 columns of C18 silica (1 X 250 mm, Applied Biosystems, Inc.), quantitative amino acid analysis and sequencing, as described above. Peptides were generally stored in the lyophilized form at 4°C prior to use in the antibacterial assays.

It is understood in the art that there are other suitable peptide synthetic devices or that manual peptide synthesis could be carried out to produce the peptides of the present invention. Automated solid phase peptide synthesis is described, e.g., in Stewart et al. (1984) *Solid Phase Peptide Synthesis*, Pierce Chemical Company, Rockford, Illinois).

Example 2. Acylation of Oligopeptides

The peptidyl resin (NH_2 form) is acylated using an acyl chloride of the fatty acid or by a symmetrical anhydride of the fatty acid until a complete acylation is achieved (using a 5-fold excess if acylating agent, in dimethyl formamide at room temperature overnight). After complete acylation is achieved, the acylated peptide is removed from the resin and deprotected as described above.

Example 3. Antimicrobial Activity Testing

Neisseria gonorrhoeae strain FA 102 and *Staphylococcus aureus* strain 8325-4 were the test bacteria used in many experiments; these strains have been described previously (Shafer et al. (1986) supra; Shafer and Onunka (1989) J. Gen. Microbiol. 135:825-830). *N. gonorrhoeae* cultures were passaged on clear typing agar as nonpiliated, transparent variants. For testing, cultures were grown with shaking at 37°C in GC broth containing glucose, iron and sodium bicarbonate supplements. *S. aureus* was grown at 37°C with shaking in LB broth. At midlogarithmic phase (OD_{550} of 0.35) the cultures were diluted in Hanks Balanced Salt Solution (HBSS) (Gibco Laboratories, Grand Island, New York) (pH 7.5) to give

approximately 10^5 CFU/ml. In other experiments, *P. aeruginosa* ATCC 27853, a standard antibiotic tester strain, was used.

Peptides were dissolved in HBSS (pH 7.5) and added in various amounts (0 to 100 micrograms) to sterile microtiter plate wells. After UV sterilization of the wells, 0.1 ml samples of the bacterial were added and the volumes in each well were adjusted with HBSS to 0.2 ml. The bacteria-peptide mixtures were incubated at 37°C for 45-60 min unless otherwise noted. For *N. gonorrhoeae*, incubation was carried out under an atmosphere of 5% CO_2. In other experiments, as noted, 1/100 strength HBSS was used. For at least some strains, the use of 1/100 HBSS resulted in greater sensitivity to the bactericidal activity of the acylated peptides disclosed herein.

Viability was determined after incubation by plating 10 and 100 microliter samples on LB agar (*S. aureus*) or GCB agar (*N. gonorrhoeae*). All assays were done in duplicate or triplicate, and the results given are the means of three independent experiments. The % survival of the test bacteria was calculated as 100 x (# CFU in the presence of peptide)/(# CFU in the absence of peptide); standard error of the mean for each data point was never greater than 5%.

Table 1. Susceptibility of Gram-Positive, Antibiotic-Resistant Bacteria to CG 117-136 and C12-Modified Peptide[1]

	MIC Values (micrograms/ml)	
Bacteria	CG 117-136	CG 117-136 C12-modified
Enterococcus faecalis		
V583 (VRE; *vanB*)	500	7.8
EU1	500	1.95
Enterococcus faecium		
C37 (VRE; *vanA*)[2]	500	1.95
UH37 (VRE; *vanB*)	250	3.9
0366 (VRE; *vanB*)	125	15.6
C67 (VRE; *vanB*)	500	3.9
Staphylococcus aureus		
COL (MRSA)[3]	1000	15.6
33593 (MRSA)	125	7.8
Michigan (VISA)[4]	1000	31.2
Streptococcus pneumoniae		
GA8904	>500	15.6
GA7797 (Ery^R)[5]	>500	15.6
GA8998 (Pen^R)[6]	>500	7.8
GA13893 (Pen^R and Ery^R)	>500	7.8

[1] Bactericidal assays were conducted as described by Shafer et al. [64] and the values reported are representative from at least three experiments.

[2] VRE, vancomycin-resistant enterococci due to the *vanA* or *vanB* genes.

[3] MRSA, methicillin-resistant *S. aureus*.

[4] VISA, vancomycin-intermediate *S. aureus*.

[5] Ery^R, erythromycin-resistant.

[6] Pen^R, penicillin-resistant

Example 4. Animal Toxicity Studies:

Female and male Balb/C mice were given IP or IV injections of increasing doses of the C12-modified CG 117-136 peptide and observed for 96 hours; the peptide had been dissolved in endotoxin-free distilled water. The lethal dose 50% (LD_{50}) for both male and female mice was determined to be at least about 150 mg/kg IP, while that for the IV injections for both genders was about 20-25 mg/kg. The highest IP dosage utilized was 150 mg/kg body weight; it did not lead to any observable adverse effects in the mice.

In order to determine whether daily injections of peptide might cause toxicity, female Balb/C mice were injected daily for 5 days with peptide IP (25 mg/kg) or for 4 days IV (10 mg/kg). Due to severe bruising of the tail veins after four days, we could not extend the IV injection past this period. Groups of mice, including control animals receiving just sterile water, were then sacrificed and a pathological analysis was performed. The animal pathologist reported that none of the mice displayed damage to any organ and that the lungs appeared normal. No adverse affects were attributed to peptide administration.

Example 5. Design of Peptides with Enhanced Bactericidal Activity:

We have designed and tested the bactericidal action of several variants of CG 117-136 that contain amino acid replacements for the methionine group at position 16 and the threonine group at position 20. Other peptides contained short (1-3 amino acids) extensions from the – and/or C-termini.

The goal is to obtain an antimicrobial peptide that remains soluble after modification with the C-12 fatty acid, retains potency against the test bacteria (*S. aureus, S. pneumoniae*, vancomycin-resistant enterococci and *P. aeruginosa*). These peptides also have a C-terminal amino acid that would permit enzymatic or chemical cleavage of peptide multimers generated during recombinant synthesis of peptide concatemers.

The amino acid sequences of the test peptides and their antimicrobial action against *P. aeruginosa* are described below; this particular strain is resistant to several antibiotics including numerous β-lactams and chloramphenicol. In this screen, we used the micro-titer

broth dilution assay of Shafer et al. [64] that measures the reduction in colony forming units (CFU) after two hours of incubation. The minimal growth inhibitory concentration (MGIC) is defined as the lowest concentration of peptide that results in no CFUs in a 2 μl sample plated onto Mueller Hinton agar.

Table 2. Susceptibility of Drug Resistant *P. aeruginosa* to Antimicrobial Peptides

Peptide (amino acid sequence)	MGIC (μg/ml)
CG 117-136 (RPGTLCTVAGWGRVSMRRGT)	31.2
CG 117-136 GER. (GTRPGTLCTVAGWGRVSMRRGTR)	15.6
CG 117-136 Furin (GTRPGTLCTVAGWGRVSMRRGTRKKR)	3.9
CG 117-136 Leu/Hse#1 (RPGTLCTVAGWGRVSLRRGHseR)	1.9
CG 117-136 Leu/Hse#2 (RPGTLCTVAGWGRVSLRRGHse)	15.6
CG 117-136 Leu/Hse#3 (RPGTLCTVAGWGRVSLRRGTRHse)	31.2
CG 117-136 Gln/Hse (RPGTLCTVAGWGRVSQRRGHse)	62.5

After initial testing results had been obtained, it was decided that it would be best to plant on using chemical (CNBr) cleavage of peptide multimers (concatemers). This necessitates the substitution of the Met-16 group with either leucine or glutamine and the presence of methionine as the C-terminal amino acid. Because homoserine (Hse) is generated after CNBr cleavage reaction, the synthetic peptide has this residue as the C-terminal amino acid. The CG 117-136 Leu/Hse#2 (RPGTLCTVAGWGRVSLRRGHse) peptide was insoluble after C-12 modification. Further studies were carried out using
CG 117-136 Leu/Hse#3 (RPGTLCTVAGWGRVSLRRGTRHse) and
CG 117-136 Gln/Hse (RPGTLCTVAGWGRVSQRRGHse).

We next examined the capacity of these two peptides and the original CG 117-136 peptide to exert bactericidal action against the strain of *S. pneumoniae* (SP) that is used the animal protection experiments (WU strain). As is shown in Table 3 below, CG 117-136 was ineffective against this organism. However, both peptides containing the Hse extension exerted antimicrobial activity against this strain. The peptide containing the Leu-16 substitution and

the 22 amino peptide with the GTRHse C-terminal sequence were at least four-fold more active than the Gln-16 peptide.

Table 3. Susceptibility of *S. pneumoniae* to Antimicrobial Peptides

Peptide	MGIC (μg/ml)
CG 117-136 RPGTLCTVAGWGRVSMRRGT	1280
CG 117-136 Gln/Hse RPGTLCTVAGWGRVSQRRGHse	320
CG 117-136 Leu/Hse#3 RPGTLCTVAGWGRVSLRRGTRHse	80

Accordingly, we sought to enhance the bactericidal activity of these CG 117-136 peptide variants through attachment of C-12 to the amino terminus. Both Hse-containing peptides retained solubility in 0.01% acetic acid with 0.2% BSA after C-12 modification. These C12-modified peptides were tested against *E. coli* (EC), *P. aeruginosa* (PA) and *S. aureus* (SA) as summarized in Table 4.

Both peptides exerted bactericidal action against all five pathogens, but the C12-modified Leu/Hse#3 peptide (RPGTLCTVAGWGRVSLRRGTRHse) appears to be the most potent acylated peptide derivative studied to date.

Table 4. Bactericidal Activity of C-12 Modified Peptides

	MGIC (μg/ml) vs.				
	PA	EC	SP	SA	EF
C12-RPGTLCTVAGWGRVSLRRGTRHse	15	10	2	8	7.5
C12-RPGTLCTVAGWGRVSQRRGHse	25	40	ND	20	ND

We next examined the killing of SP strain WU2 over a one-hour incubation period by C12-RPGTLCTVAGWGRVSLRRGTRHse (C12-CG 1176-136 Leu/Hse#3) in an incubation fluid consisting of Mueller Hinton broth (MHB) or Todd Hewitt broth (THB); the former is the standard broth used in bactericidal assays, while the latter is the preferred broth for

growing SP. The MGIC of the peptide for SP incubated in MHB was 3 μg/ml while in THB it was 4 μg/ml.

Thus, the strain of SP used in these studies is very sensitive to the lethal action of C12-RPGTLCTVAGWGRVSLRRGFTRHse.

We then examined the ability of peptide C12-RPGTLCTVAGWGRVSLRRGTRHse (C12-CG 117-136 Leu/Hse#3) to kill VRE isolates with either *vanA* or *vanB* genotypes. We used the bactericidal assays described above and the conventional microbroth dilution assay of the NCCLS, which measures the minimal inhibitory concentration (MIC) of antibiotics after 24 hours of incubation of the test bacteria at 37°C. The differences between the MGIC and MIC values likely represent incomplete killing after two hours (the incubation period for the MGIC assay). Nevertheless, the results showed that in both assays, all four VRE strains were, to varying degrees, susceptible to the action of the peptide.

Table 5. Susceptibility of VRE Isolates to C-12-modified Leu/Hse#3 Peptide (C12-RPGTLCTVAGWGRVSLRRGTRHse)

Strain	Van genotype	MGIC (μg/ml)	MIC (μg/ml)
C37	*vanA*	16	64
C68	*vanB*	1	4
UH37	*vanB*	4	32
99	*vanB*	2	32

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WE CLAIM:

1. An acylated oligopeptide having antimicrobial activity, wherein said oligopeptide comprises a sequence selected from the group consisting of RPGTLCTVAGWGRVSMRRGT (amino acids 117-136 of SEQ ID NO:1), IIGGRESRPHSRPYMAYLQI (amino acids 1-20 fo SEQ ID NO:1) SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7. SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and D-enantiomers of any of the foregoing, and wherein said peptide is acylated with a fatty acid comprises from about 2 to about 20 carbon atoms and wherein said peptide is acylated at either the N-terminus or the C-terminus.

2. The acylated oligopeptide of claim 1 wherein the fatty acid comprises from 8 to about 14 carbon atoms.

3, The acylated oligopeptide of claim 1 wherein the fatty acid is a saturated fatty acid.

4. The acylated oligopeptide of claim 1 wherein the fatty acid is a straight chain fatty acid.

5. The acylated oligopeptide of claim 4 wherein the fatty acid is lauric acid.

6. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:1, amino acids 117-136.

7. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:2.

8. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:3.

9. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:4.

10. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:5.

11. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:6.

12. The acylated oligopeptide of claim 1 wherein said oligopeptide comprises an amino acid sequence as given in SEQ ID NO:7.

13. A therapeutic composition suitable for controlling infection by a bacterium, said composition comprising at least one acylated antimicrobial oligopeptide of claim 1 and a pharmacologically acceptable carrier, wherein the bacterium is sensitive to the antimicrobial activity of at least one of said oligopeptides.

14. A method for controlling infection by a bacterium, said method comprising the step of administering a therapeutically effective amount of the therapeutic composition of claim 13.

15. A method of inhibiting growth of a bacterium, said method comprising the step of exposing a bacterium to at least one acylated antimicrobial oligopeptide of claim 1 in an amount effective for reducing viability of said bacterium at least by 90%, wherein the bacterium is sensitive to the antimicrobial activity of said at least one acylated antimicrobial oligopeptide.

1 61
IIGGRESRPHSRPYMAYLQIQSPAGQSRCGGFLVREDFVLTAAHCWGSNINVTLGAHNIDRRENTQ

80 117
QHITARRAIRHPQYNQRTIQNDIMLLQLSRRVRRNRNVNPVALPRAQEGLRPGTLCTVAGWGRVS

136
MRRGTDTLREVQLRVQRDRQCLRIFGSYDPRRQICVGDRRERKAAFKGDSGGPLLCNNVAHGIVS

223
YGKSSGVPPEVFTRVSSFLPWIRTTMR

FIG. 1

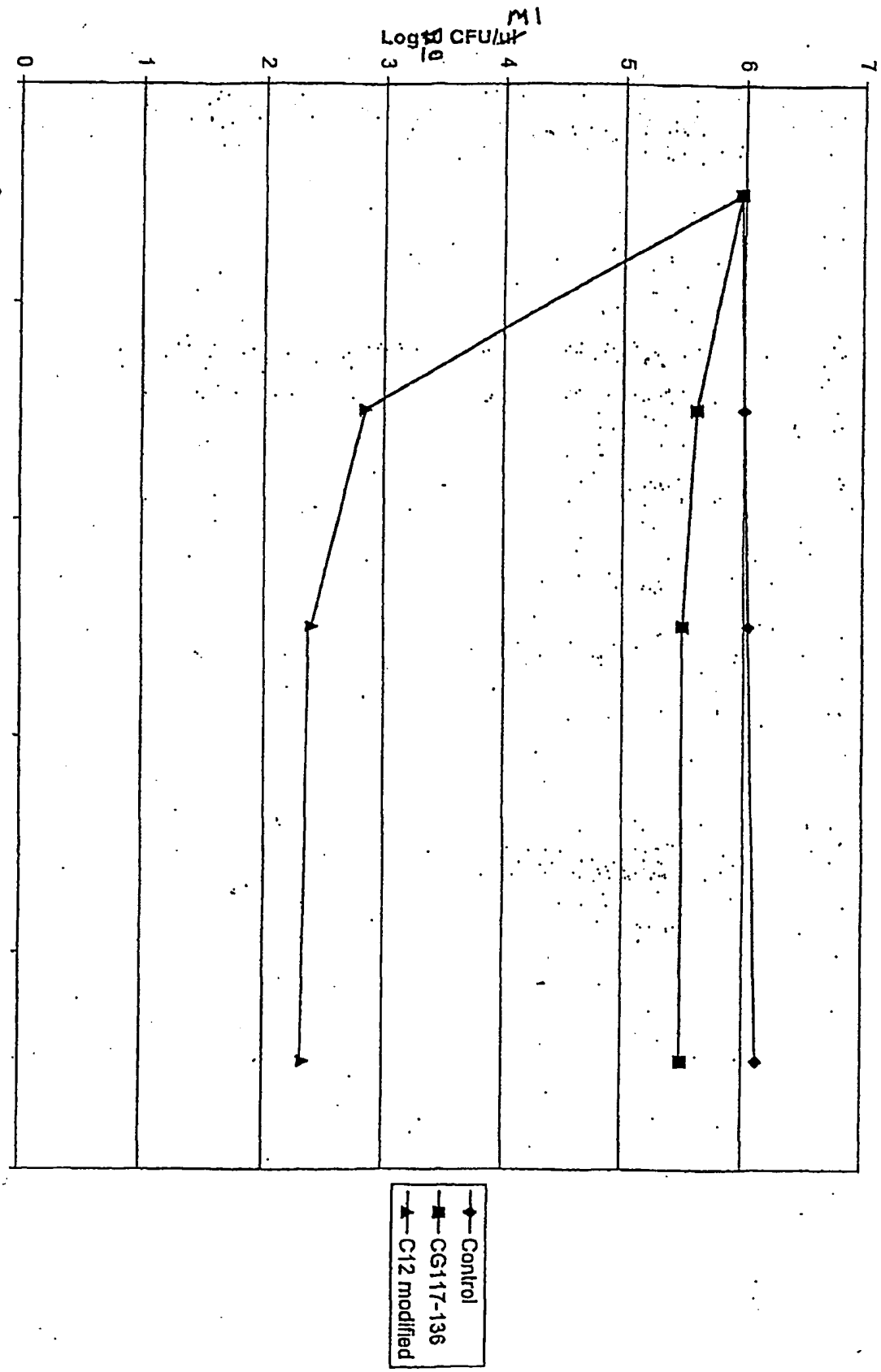
Log10 CFU/ml
0
1
2
3
4
5
6
7
0
30
60
120
Minutes
Control
CG117-136
C12 modified

FIG. 2

SEQUENCE LISTING

<110> Emory University

<120> Acylated Antimicrobial Peptides

<130> 119-01WO

<140> Not assigned
<141> 2001-11-15

<150> US 60/248,803
<151> 2000-11-15

<160> 14

<170> PatentIn Ver. 2.0

<210> 1
<211> 224
<212> PRT
<213> Homo sapiens

<400> 1

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 1               5                  10                  15

Tyr Leu Gln Ile Gln Ser Pro Ala Gly Gln Ser Arg Cys Gly Gly Phe
            20                  25                  30

Leu Val Arg Glu Asp Phe Val Leu Thr Ala Ala His Cys Trp Gly Ser
        35                  40                  45

Asn Ile Asn Val Thr Leu Gly Ala His Asn Ile Asp Arg Arg Arg Glu
    50                  55                  60

Asn Thr Gln Gln His Ile Thr Ala Arg Arg Ala Ile Arg His Pro Gln
65                  70                  75                  80

Tyr Asn Gln Arg Thr Ile Gln Asn Asp Ile Met Leu Leu Gln Leu Ser
                85                  90                  95

Arg Arg Val Arg Arg Asn Arg Asn Val Asn Pro Val Ala Leu Pro Arg
            100                 105                 110

Ala Gln Glu Gly Leu Arg Pro Gly Thr Leu Cys Thr Val Ala Gly Trp
        115                 120                 125

Gly Arg Val Ser Met Arg Arg Gly Thr Asp Thr Leu Arg Glu Val Gln
    130                 135                 140

Leu Arg Val Gln Arg Asp Arg Gln Cys L u Arg Ile Phe Gly Ser Tyr
145                 150                 155                 160
```

```
Asp Pro Arg Arg Gln Ile Cys Val Gly Asp Arg Arg Glu Arg Lys Ala
                165                 170                 175

Ala Phe Lys Gly Asp Ser Gly Gly Pro Leu Leu Cys Asn Asn Val Ala
            180                 185                 190

His Gly Ile Val Ser Tyr Gly Lys Ser Ser Gly Val Pro Pro Glu Val
        195                 200                 205

Phe Thr Arg Val Ser Ser Phe Leu Pro Trp Ile Arg Thr Thr Met Arg
    210                 215                 220
```

<210> 2
<211> 23
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 2

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  1               5                  10                  15

Ser Met Arg Arg Gly Thr Arg
            20
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<210> 3
<211> 26
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<220>
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  1               5                  10                  15

Ser Met Arg Arg Gly Thr Arg Lys Lys Arg
            20                  25
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<210> 4
<211> 21
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<220>
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<220>
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<222> (20)
<223> X at position 20 is homoserine.

<400> 4
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1 5 10 15

Arg Arg Gly Xaa Arg
20

<210> 5
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
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<220>
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<222> (20)
<223> X at position 20 is homoserine.

<400> 5
Arg Pro Gly Thr Leu Cys Thr Val Ala Gly Trp Gly Arg Val Ser Leu
1 5 10 15

Arg Arg Gly Xaa
20

<210> 6
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<220>
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<223> X at position 22 is homoserine.

<400> 6
Arg Pro Gly Thr Leu Cys Thr Val Ala Gly Trp Gly Arg Val Ser Leu
1 5 10 15

Arg Arg Gly Thr Arg Xaa
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<210> 7
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Ser Met Arg Arg Gly Thr Arg Lys
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Ser Met Arg Arg Gly Thr Arg Lys Lys Arg Lys
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1 5 10 15

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<210> 14
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Arg Arg Gly Thr Lys
20